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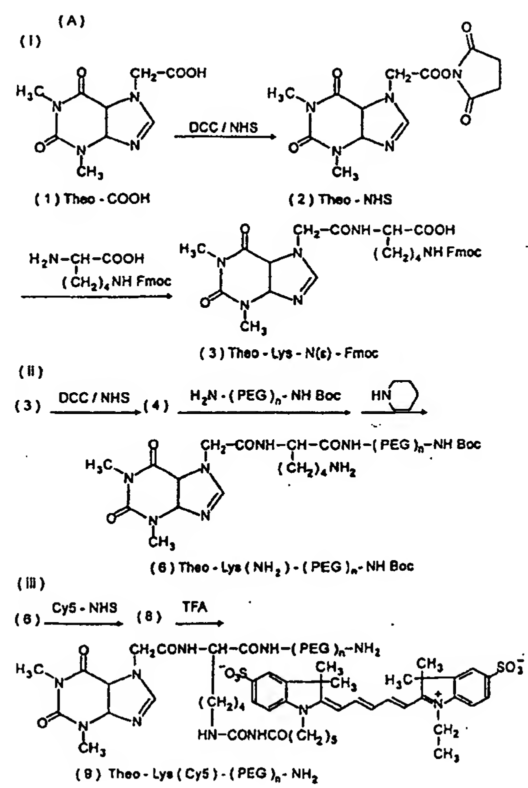
(57) Abstract

The present invention concerns a sensor for detection of a biological analyte in a test sample. The biological analyte is a member of a pair forming group such as antigen/antibody, ligand/receptor, etc. The sensor comprises a porous matrix where in each cavity are entrapped a molecule capable of specifically binding to the analyte as well as an analyte-analogue. Competitive displacement of the analyte-analogue by the assayed analyte bring to chahge at least one detectable property of the sensor.



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SENSORS

FIELD OF THE INVENTION

The present invention is in the field of sensors, particularly biosensors for the detection of an analyte in a test medium.

More specifically, the present invention concerns a sensor comprising a porous matrix.

BACKGROUND OF THE INVENTION

Chemical and biochemical sensors have a great potential for application in fields such as medicine, industry and defense. In medicine, biosensors are useful, for example, for monitoring, clinically and biochemically important analytes both *in vivo* and *in vitro*. For *in vivo* monitoring, sensors having the capacity of continuous determination of the analyte are particularly desired. Sensors for continuously monitoring analytes in real time are also of great importance in industry for controlling and regulating chemical process as well as for monitoring biological and biochemical hazards.

U.S. Patent 5,156,972 concerns an analyte–specific sensor for determining an analyte in a test sample. The sensor comprises a sensing surface to which there are linked a plurality of reversible competitive recognition units (RCRU). Each such unit contains at least one receptor and at least one ligand, one of which is an analyte–analogue. In these RCRUs the receptor and ligand are *a priori* connected to each other, directly or indirectly, in such a configuration that even when the analyte–analogue ligand is displaced from the receptor by an analyte ligand, the analogue is still retained in close proximity to the receptor. The relative positions of the receptor and ligand in each RCRU is such that when no analyte is present in the sample, or when the analyte concentration is low, the receptor and the ligand affinity bind to each other. The fluctuations in the analyte concentration in the test sample affect the chemical occurrences of the RCRU, and consequently the characteristics of the sensor. The changes in the analyte concentrations are monitored continuously.

The advantage of the biosensor of said U.S. patent, as compared to prior art biosensors, resides in the fact that the sensor is truly reversible due to the fact that the receptor and the analogue are *a priori* bound to each other. This means that even when the receptor is bound to the analyte, the analyte–analogue stays in a closed vicinity to the bound receptor, so that when the analyte concentration drops again, the analyte–analogue may again bind to the receptor. The reversible nature of this sensor enables continuous, on–line monitoring of the analyte concentration in the sample.

Some biosensors are based on sol–gel encapsulation methods, wherein proteins are entrapped within a silicate glass matrix prepared by a sol–gel method. Research has revealed that silicate glass obtained by the sol–gel method can thus provide a host matrix so that biomolecules immobilized in this matrix retain their functional characteristics to a large extent. Moreover, these functionalized glasses can be prepared so that they are optically transparent permitting optical monitoring of the spectrometric properties of the encapsulated biomolecules (Dave *et al.*, *Analytical Chemistry*, <u>66</u>(22):1120–1127, (1994)).

Enhanced evanescent wave sensors have been prepared based on sol-gelled-derived porous glass coating (MacCraith, *Sensors and Actuators B*, 11:29-33, (1993)), as well as optical sensing platforms based on a combination of planar waveguiding and sol-gel processing technologies (Yang *et al.*, *Analytical Chemistry*, 67:1307-1314, (1995)).

One of the proteins which was previously encapsulated within a sol-gel prepared silicate glass matrix is an antibody, such as antifluorescein, and it has been demonstrated that the sol-gel-encapsulated antibody retains its affinity for fluorescein although the encapsulation process decreased the affinity constant by about two orders of magnitude (Wang *et al.*, *Anal. Chem.*, 65:2671-2677 (1993)). The advantage of such encapsulated antibodies, resides in the fact that they are relatively unapproachable to proteases, and thus, generally speaking, biosensors comprising an antibody entrapped within a transparent sol-gel glass are stable for long periods of time. However, the publication of Wang *et al.* discloses a biosensor based on non-competitive binding of an antigen to an antibody, and not on competitive displacement of an analyte-analogue bound to an antibody by the analyte. Furthermore, the biosensor of Wang *et al* is not reversible and thus cannot enable on-line continuous monitoring of an analyte in a test medium.

GLOSSARY

The following are the meaning of some of the terms used in the description and claims herein.

Analyte - a molecule which presence is to be assayed or its concentration is to be determined in the test medium. The analyte is a first member of *a pair forming group* (see below).

Pair forming group - two molecules which have the capability of specifically recognizing and *specifically binding* (see below) to each other.

Typical examples are antibody/antigen; receptor/ligand; lectin/glycoprotein; enzyme/substrate; two complementary strands of nucleic acid sequences and the like.

Specific binding - non-covalent affinity binding between two members of a pair-forming group.

Matrix having a plurality of porous cavities - a solid matrix having within a plurality of spaces (cavities), some of said cavities are in communication with the ambient surrounding through pores present at the surface of the matrix, which cavities are termed *"porous cavities"*.

Second member of pair forming group - where the analyte is one member of the pair forming group this term refers to the other member of the same specific group, for example, where the analyte is an antigen, the second member is its specific antibody, where the analyte is a specific nucleic acid sequence, the second member is the complementary sequence, etc.

Analyte-analogue - a component of the *detecting unit* (see below) or the *detecting pair* (see below) which is capable of specifically binding to the second member of the pair forming group.

Detecting unit - a single unit in accordance with the first embodiment of the invention composed of the second member of the pair forming group *linked* (see below) to the analyte-analogue in such an orientation that specific binding between the two is possible.

Detecting pair - two separate molecules, one being second member of the pair forming group and the other being the analyte-analogue. The two molecules are entrapped within the same cavity of the matrix having a plurality of porous cavities.

Link – a strong bond between the second member of the pair forming group and the analyte–analogue which like results in the detection unit. The link is formed in such an orientation that enables specific binding between the analyte–analogue and the second member. The link may be a covalent bond, or may be achieved by utilization of moieties having an exceptionally high affinity towards each other, such as through the biotin/avidin binding.

First pair – a state where the second member and the analyte are specifically bound to each other.

Second pair – a state where the second member and the analyte–analogue are specifically bound to each other.

Medium – a liquid which holds within it the analyte to be assayed. The analyte may be present in the liquid, *a priori*, or may be initially present in a gaseous or solid phase. For detection purposes, the gaseous or solid phase are contacted with the liquid in order to dissolve the analyte therein. The medium is brought into contact with the matrix having a plurality of porous cavities, so that it may enter each cavity and carry the analyte to the detecting unit or detecting pair present therein.

Signal producing moiety – compounds which are able to change a detectable signal as a result of transition between the state of the first pair and the state of the second pair.

SUMMARY OF THE INVENTION

The present invention concerns a sensor for detecting an analyte in a test medium. In accordance with the first embodiment of the invention termed *"the unit embodiment"*, the sensor is composed of a matrix having a plurality of porous cavities in which there are entrapped a plurality of detecting units each composed of a member capable of specifically binding to the analyte (termed hereinafter *"the second member of the pair forming*

to the analyte (termed hereinafter *"the second member of the pair forming unit"* or *"second member"*) linked to an analyte–analogue which can specifically bind to the second member. The linking is such as to allow the second member and the analyte–analogue to specifically bind to each other in a competitive reversible manner. The analyte may competitively displace the analyte–analogue to bind specifically to said second member. Since the analyte–analogue is linked to the second member, it remains in the vicinity of the second member even when the second member is bound to the analyte. If the analyte's concentration decreases, the analyte–analogue may again specifically bind to the second member, so that the detecting units of the sensor of the invention are truly reversible.

In accordance with the second embodiment of the invention termed *"the pair embodiment"* the sensor is composed of a matrix of porous cavities and in each cavity is entrapped a pair of two molecules, one being the second member of the pair forming group and the other being the analyte–analogue, both molecules together are termed *"a detecting pair"*. In a similar manner to the one described above, in the absence of the analyte, the analyte–analogue is specifically bound to the second member in a competitive–reversible manner. The analyte may competitively displace the analyte–analogue to bind specifically to the second member. According to the pair embodiment, the analyte–analogue and the second member are in the vicinity of each other, not due to a physical link between them, but rather due to the fact that both are confined to the small space of the cavity.

The transition of the second member, in both embodiments of the invention, from a state of binding to the analyte–analogue to a state of binding the analyte and *vice versa* changes at least one detectable property of the sensor. Thus determination or measurement of said change is indicative to the presence or concentration, respectively, of the analyte in the test medium.

The pores of said matrix have properties which allow the analyte molecule to pass to the entrapped detecting units or detecting pairs, while these properties eliminate the passage of the detecting unit (in the unit

embodiment of the invention) or each molecule of the detecting pair (in the pair embodiment of the invention) therethrough. Generally speaking, the properties are size and/or electrical charge of the pores.

The detecting units or detecting pairs of the invention feature all the advantages of the so called *"reversible competitive recognition units"* (RCRUs) as specified in U.S. 5,156,972 which are: specificity to the analyte and reversibility of the detection assay, which reversibility allows for on-line continuous determination of analyte presence or of the analyte concentration in a test medium.

However, in the present invention, the detecting units or detecting pairs are not immobilized on a solid support as disclosed in U.S. 5,156,972 but rather are entrapped within the porous cavities of the matrix. Said entrapment eliminates the need to immobilize each detecting unit or each molecule of the detecting pair to the support so in accordance with the present invention the process of preparation of the sensor is simplified.

Furthermore, the pores of the matrix are typically too small to allow entrance of proteolytic enzymes to the entrapped detecting units or the entrapped molecules of the detecting pair. Thus, in the sensor of the invention, the detecting units or detecting pairs are protected from degradation by various enzymes and consequently the sensor of the invention is stable for substantially longer periods of time than the sensor of U.S. 5,156,972.

Thus the present invention provides a sensor for the detection of an analyte in a test medium, the analyte being a first member of a pair forming group, the sensor comprising:

a matrix having a plurality of porous cavities, entrapping within them detecting units, wherein the pore properties of the cavities are such that the detecting units are entrapped within the cavities while the analyte may move freely in and out of the cavities; each of the detecting units comprising the second member of the pair forming group linked to an analyte-analogue which can specifically bind to said second member in a reversible manner; the linking being in a manner enabling specific binding between said second

member and said analyte–analogue; specific binding between the analyte and said second member giving rise to the formation of a first pair and specific binding between said analyte–analogue and said second member giving rise to the formation of a second pair; transition between said first pair and said second pair resulting in a change in at least one detectable property in said sensor.

The present invention further provides a sensor for the detection of an analyte in a test medium, the analyte being a first member of a pair forming group the sensor comprising:

a matrix having a plurality of porous cavities, a single cavity entrapping within it essentially a single detecting pair, wherein the pore properties of the cavities are such that the molecules of the detecting pair are entrapped within the cavities, while the analyte may move freely in and out of the cavities; one molecule of the detecting pair comprises the second member of the pair forming group, and the other molecule of the detecting pair comprises an analyte–analogue which can specifically bind to said second member in a reversible manner; specific binding between the analyte and said second member giving rise to the formation of a first pair and specific binding between said analyte–analogue and said second member giving rise to the formation of a second pair; transition between said first pair and said second pair resulting in a change in at least one detectable property in said sensor.

The ratio of the first pair to the second pair correlates to the concentration of the analyte in the test sample. Since the level of the detectable property reflects said ratio it may be calibrated to indicate the concentration of the analyte in the test medium.

The present invention further provides an apparatus for detecting of an analyte in a test sample comprising the sensor of the invention and means for determining or measuring the change in said at least one detectable property of the sensor.

The apparatus of the invention may further comprise a signal producing moiety which is capable of transducing the transition between the

first pair and the second pair and *vice versa* to a detectable property. Examples of signal producing moieties are moieties which change their optical properties (for example refractive index or luminescence), electro-chemical properties or electro chemiluminescence properties as a result of transition between the first and second pair.

Due to the reversible nature of the competitive interactions of the detecting units of the sensor of the invention, both the sensor and the apparatus of the invention are suitable for continuous monitoring of an analyte in a liquid.

GENERAL DESCRIPTION

The analyte to be determined in accordance with the present invention is a first member of a pair forming group such as an antibody, or an antigen; an enzyme or its substrate; a ligand (such as a hormone, drug, peptide, etc.) or its soluble receptor; one strand of a nucleic acid sequence and the like.

The test medium containing the analyte should be a liquid medium. The analyte may be present initially in the liquid, for example in a body fluid sample, or may initially be present in a solid or gaseous medium and then dissolved in the liquid which is assayed. Alternatively, the porous sol-gel matrix may be loaded prior to the determination with a suitable liquid, and a gaseous sample is then passed on the porous sol-gel matrix so that the analyte present in the gas is dissolved in the liquid already present within the cavities of the sensor's matrix.

The sensor of the invention is capable of determining the presence or absence of an analyte in the test medium in a binary fashion by determining whether a change in the detectable properties did or did not take place. Alternatively, it is possible to determine the concentration of the analyte in the test sample by measuring the amount change in the detectable property and calibrating said measurement.

The term *"matrix having a plurality of porous cavities"* refers to a matrix having a plurality of spaces (cavities) within, some of the spaces

being in communication with the ambient surrounding through pores which are present at the surface of said matrix. The properties of the pores should be such which entrap within the cavity the detecting units (in the unit embodiment) or the detecting pair (in the pair embodiment) while enabling the analyte to more freely in and out of the cavity. The property of the pore which discriminates between the analyte and the detecting unit or each molecule of the detecting pair is based usually on the pore's size. However, other pore properties such as an electric charge may also be used for discrimination purposes.

Essentially one detecting unit or one detecting pair are entrapped within each cavity of the matrix due to the manner of the preparation of the matrix as will be explained hereinbelow. Where the pair embodiment of the invention is utilized, typically one molecule of the second member and one molecule of the analyte-analogue are entrapped within each cavity. This occurs since due to the affinity between these two molecules, they form specific binding pairs already in the reaction medium, which pairs are then entrapped wtihin the pores. A typical example of such a porous matrix is silicate glass, produced by a sol-gel method which proceeds by hydrolysis of the monomeric precursors of the matrix followed by polycondensation of the hydroxylated units to form a porous gel. The detecting units or detecting pairs can be incorporated to the undensified sol-gel glass by adding it to the gel before gelation, substantially as described in Wang *et al., Anal. Chem.* 65:2672-2675 (1993)). Examples of possible matrices beyond silicate glass are cellulose nitrate, sodium alginate, polyacrylamide, agarose, dextran and gelatin.

The detection unit according to the unit embodiment of the invention comprises the second member of the pair forming group linked to an analyte-analogue. The detecting pair, according to the pair embodiment of the invention comprises two separate molecules, one being the second member of the pair forming group and the other being the analyte-analogue. The second member may be the full molecule, for example, where the analyte is a ligand or an antigen, the second member is the full receptor or

antibody, respectively, or alternatively may be a ligand binding fragment or an antigen binding fragment of the receptor or the antibody, respectively.

The analyte-analogue is also a member of a pair forming group which is capable of specifically binding to said second member. The analyte-analogue may have the same binding affinity to the second member as that of the analyte. However it is preferable that the affinity of the analyte-analogue to the second member will be lower than that of the analyte, in order to compensate for the fact that the analyte-analogue effective concentration near the second member is very high (since both are confined to the same cavity). The analyte-analogue may be the molecule of the analyte itself, chemically modified derivatives of the analyte, fragments of the analyte (for example, antibody binding fragments of an antigen where the analyte is an antigen) or a molecule which is completely different than the analyte., but competes with the analyte for binding to the same binding site of the second member. When the pair embodiment of the invention is utilized, it is preferable that the analyte-analogue is considerably larger in size than the analyte, so that it is entrapped within the cavity while the analyte can pass freely in and out of the cavity. This can be achieved by conjugating a molecule similar to the analyte to a large inert moiety such as a bead or a molecule of human serum albumin (HSA).

The second member and the analyte-analogue are linked to each other in the unit embodiment of the invention. The link may be a covalent linkage achieved by attaching a linker to regions of the second member and of the analyte-analogue which are not involved in the specific binding, in such a manner so that the linker does not interfere with said specific binding. The linker is of a length and structure which allows under appropriate conditions, (such as absence or decrease of analyte concentration), specific binding between the second member and the binding-moiety. Examples of such linkers which cause covalent binding are polypeptidic chains such as polyalanine or chains of polyethyleneglycol, and the like. Alternatively, the linkage between the analyte-analogue and the second member may be non-covalent, for example a link mediated by moieties

having exceptionally high affinity to each other such as biotin and avidin. In the latter case it is possible to attach to the second member, through a suitable spacer, to biotin and attach to the analyte–analogue, through a spacer, to avidin (or *vice versa*) and then allow these two moieties to interact thus linking the second member and the analyte–analogue to each other.

Since the ratio of the second member to the analyte–analogue is constant, the proportion of second members which are bound to the analyte–analogue at any given condition to form the second pair is dependent on the concentration of the analyte inside the cavity, which concentration is dependent on the concentration of the analyte in the medium.

The detectable properties of the sensor which are changed as a result of binding or dissociation of the analyte to the second member may be photochemical, e.g. light absorption, light emission, light scattering and light polarization or electrochemical, electrochemiluminescence or piezoelectrical properties.

In accordance with one embodiment of the invention the detectable property is photochemical.

Generally, the physical characteristics of light which is emitted by a fluorescent labelled analyte–analogue or a labeled second member maybe designed to be different where the two are bound or unbound to each other. Accordingly the changes in the concentration of analyte may be monitored by observing the alterations of the luminescent emission, caused by association or dissociation of the analyte–analogue to said second member.

One way of causing and detecting luminescence in a sensor according to the invention, is to design the matrix as part of a waveguide so that the evanescent light wave propagating at the solid phase/liquid or gaseous phase interface excites the luminophore binding–moiety or a luminophore second member and the emitted luminescence is conducted via the waveguide to an appropriate detector, as described in MacCraith, B.D., *Sensors and Actuators*, B11:29–39 (1993).

In case the sensing is based on measurement of fluorescence intensity the distinction between the first pair state and the second pair state may be sharpened by fitting each second member (e.g. receptor), of the detecting unit or detecting pair with a luminescence quencher group, for example, Rhodamine, while the analyte–analogue (e.g. ligand), contains a fluorescein group or *vice versa*. In this way, luminescence in the state of the second pair wherein the analyte–analogue is specifically bound to the second member (which occurs in the absence of analyte, or in low concentrations thereof), is eliminated or reduced due to the energy transfer between the luminophore group of the labelled analyte–analogue and the quencher group of the receptor. When the fluorophore labelled analyte–analogue is displaced from the second pair by a competing analyte, the energy transfer between the group of the labelled analyte–analogue and the quencher group of the receptor is reduced, and as a result the luminescence increases. Similarly, each detecting unit or one molecule of the detecting pair may be fitted with a luminescence enhancer, and in such a case, the luminescence increases upon formation of the second pair and decreases upon its dissociation to form the first pair.

In accordance with another embodiment of the present invention, the detectable property is electrochemical.

It is well known that many compounds change their redox potential as a result of binding and dissociation to other compounds. This property may be measured by using a suitable electrode, and may be indicative as to whether the first or second pair are formed in the sensor of the invention.

Preferably, either the analyte–analogue or the second member should be fitted with a transducer which is a molecule which redox potential is substantially altered when the two are bound as compared to a situation of when the two are dissociated.

An example of an electrochemical change due to binding and dissociation is that of nitrated derivatives of estriol (Konyves, I, and Olsson, A., Acta, *Chemica Scandinavica*, <u>18</u>:483–487 (1964)) or by utilizing biotin

labeled with an electroactive labeling group (daunomycin), which redox potential is altered as a result of binding of biotin to avidin (Sugawara *et al.*, *Anal. Chem.*, 67:299-302 (1995)).

Alternatively, it is possible to use as signal producing moieties compounds which are capable of electro-generated chemilumines, i.e., can change their level of high emittance due to change in electrochemical properties, which change in properties is due to change in redox potential due to binding or dissociation (Yang *et al.*, Bio/Technology 12:193-194 (1994)).

Due to the reversible nature of the detecting units or the detecting pairs of the sensor according to the invention, there is a real time response to fluctuations of analyte concentrations in the test medium with concomitant continuous modulations of the detected property which are recorded by suitable detection instrumentation, for example of the kind described by Sutherland *et al., J. Immunol. Meth.*, 74:253-265 (1984), Tromberg *et al., Anal. Chem.*, 59:1226-1230 (1987); Bush and Rechnitz *Anal. Lett.* 20(11):1781-1790 (1987); Lakowicz and Maliwan, *Anal. Chem-Acta*, 271:155-164, (1993) and Yang *et al., supra*).

The invention also provides an apparatus for determining or measuring the concentration of an analyte in a medium, comprising the sensor of the invention and a detector capable of determining or measuring the change in the detectable property. If desired, such apparatus may be designed as monitor for continuous operation with said probing vessel being adapted for the continuous throughflow of the test medium.

BRIEF DESCRIPTION OF THE DRAWING

Figs. 1 and 2 are various formulae and reaction schemes relating to preparation of the detecting units suitable for optical detection;

Figs. 3 and 4 are various formulae and reaction schemes relating to the preparation of detecting units suitable for electrochemical detection.

DETAILED DESCRIPTION OF THE INVENTION

In the following, the numbers in brackets refer to the product numbers in Figs. 1 to 4. The following are some abbreviations which will be used throughout the following description.

Abbreviations:

DCC – N,N'–dicyclohexylcarbodiimide
NHS – N–hydroxysuccinimide
Fmoc – 9–Fluorenylmethyloxycarbonyl
BOC – t–Butyloxycarbonyl
PEG – Polyethyleneglycol
DTT – Dithiothreitol
TFA – Trifluoroacetic acid
SMBS – m–Maleimidobenzoyl–N–hydroxysulfosuccinimide ester
DSC – N,N'–Disuccinimidyl carbonate
DAUN – Daunomycin

Procedures

Example 1: Preparation of Detecting unit comprising a theophylline analyte–analogue and an antibody fragment suitable for optical detection (Figs. 1 and 2)

(A) Preparation of labeled analyte–analogue with long–spacer

(i) Product (3) (Fig. 1): was obtained by conjugation of lysine to the theophylline analogue. The preparation of product (3) was carried out in a similar procedure to those described by Parini *et al.*, *Stroids*, 46:903–913 (1985). The procedure included carboxy activation of theophylline–7–acetic acid (product 1), by using the well known reagent combination of N,N'–dicyclohexylcarbodiimide (DCC) and N–hydroxysuccinimide (NHS). The carboxy activated product (product 2) was then reacted with N(ε)–Fmoc–Lysine, the get product 3.

(ii) Product (6) (Fig. 1): was obtained by conjugation of long spacer to product (3). Product (6) was obtained by conjugation of product (3) to N(ω)–Boc–protected polyethylene glycol diamine (H_2N–$(PEG)_n$–NH–Boc). Also this conjugation was done in a similar procedure as those described by Pariani *et al., supra* to get product (5). The Fmoc protecting group, in product (5), was then removed by piperidine to get product (6).

(iii) Product (9): Conjugation of Cy5 (which serves as the fluorofor agent) to product (6). Product (6) was reacted with N–hydroxy–succinimide–Cy5 (7) under the similar conditions described by Parini *et. al., supra* to get product (8). Product (9) is then obtained by removing the Boc protecting group of product (8), and redissolving the product in a phosphate buffer solution, pH 7.5.

(B) <u>Preparation of Fab fragment of anti–theophylline (product (10) (Fig. 2)</u>

The Fab fragment was prepared from monoclonal anti–theophylline IgG, following the procedure described by Prisyazhnoy *et al., J. Chromatag.* <u>424</u>:243–253 (1988). The procedure includes digestion of the intact antibody by pepsin to get the $F(ab')_2$ and the $F(ab')_2$ was reduced further by dithiothreitol (DTT) in the presence of EDTA to get product (10).

(C) <u>Product (12) (Fig. 2): Conjugation of the labeled analyte–analogue to the Fab fragment</u>

The product (12) was obtained by crosslinking (using SMBS crosslinker) the Fab fragment (10) with the labeled analyte–analogue (11). This preparation was done in a similar way described by Jiang *et al., J. Immunolog, Methods* <u>134</u>:139–149 (1990). (X) in the figure represents long spaces.

Example 2: Preparation of an electrochemical detecting unit comprising a theophylline analyte-analogue and a Fab antibody fragment (Figs. 3 and 4)

A. Preparation of analyte-analogue with long-spacer and labeled with electrochemical active group

(i) Product (6) - Theophylline analyte-analogue with PEG long-spacer was obtained as described in Example 1.

(ii) Product (16) (Fig. 3) - Conjugation of N-hydroxysuccinimide group to daunomycin.

Daunomycin dissolved in DMF, was reacted with N,N-disuccinimidyl carbonate under a similar condition described by K. Takeda *et al.*, Tetrahedron Lett. 24:4569 (1983).

(iii) Product (18) (Fig. 3) - Conjugation of the theophylline analyte-analogue (6) to the carboxy activated daunomycin (16) is obtained by reacting product (6) with product (16) under similar conditions as described by Parini *et al., (supra)* to get product (17).

Product (18) is then obtained by removing the BOC protecting group of product (17) and redissolving the product in a phosphate buffer solution, pH 7.5.

(B) Preparation of Fab fragment of anti-theophylline (product (10))

The procedure was carried out as described in Example 1(B).

(C) Product (20) (Fig. 4) - Conjugation of the daunomycin labeled theophylline analyte-analogue to the Fab fragment

This procedure was carried out as described in Example 1(C).

Example 3:

Entrapment of the recognition units in the porous matrix

(i) The Sol-Gel Process - in brief description

The sol-gel process is a method of preparing glasses and ceramics at low temperature by hydrolysis and polymerization of organic precursors. The process typically involves a metal alkoxide, water, a solvent and frequently a catalyst, which are mixed thoroughly to achieve homogeneity on a molecular scale.

Chemical reactions (hydrolysis and condensation polymerization) lead to the formation of a viscous gel, which is an amorphous porous material containing liquid solvents in the pores. Low-temperature (typically <100°C) curing expels most of the liquids and leaves the porous oxide.

The generic precursor solution for sol-gel-derived silica may be represented by: $Si(OR)_4 + H_2O + ROH$ + acid/base catalyst, where R is an alkyl group.

(ii) The process for Sol-Gel Film Preparation, encapsulated with the detecting units of Examples 1 and 2

The silica sol was prepared following the procedure described by Ellerby *et al.*, *Science*, 225:1113-1115 (1992). Briefly, 10 g of tetramethylorthosilicate (TMOS) was mixed with 2.5 ml of deionized water, followed by the addition of 150 μl of 0.04 M HCl, and the reaction mixture was sonicated for 20 mins.

The coating solution was prepared by mixing 2 ml of sonicated sol with 2 ml of buffer solution (0.01 M sodium phosphate, pH 6.0) and 1 ml of 10 μM of theophylline-antitheophylline detecting units (prepared as described above in Example 1 or 2) in 0.01 M sodium phosphate, pH 6.0 solution.

For the purpose of preparation of an optical sensor prior to coating the waveguide (glass microscope) was precleaned by sonication for 30

mins. in a 2% (v/v) aqueous solution of PCC-54 (Pierce) rinsed in deionized water and dried overnight at 150°C.

(iii) Preparation of the sol-gel film on the planar waveguide:

About 2 ml of the coating solution was applied to the slide, followed by spinning at 1500 rpm for 2 mins. The gelation occurred within several minutes. The sol-gel film on the waveguide, encapsulated with detecting units was then stored at 4°C until used.

CLAIMS:

1. A sensor for the detection of an analyte in a test medium, the analyte being a first member of a pair forming group, the sensor comprising:

a matrix having a plurality of porous cavities entrapping within them detecting units; wherein the pore properties of the cavities are such that the detecting units are entrapped within the cavities while the analyte may move freely in and out of the cavities; each of the detecting units comprising the second member of the pair forming group linked to an analyte–analogue which can specifically bind to said second member in a reversible manner; the linking being in a manner enabling specific binding between said second member and said analyte–analogue; specific binding between the analyte and said second member giving rise to the formation of a first pair and specific binding between said analyte–analogue and said second member giving rise to the formation of a second pair; transition between said first pair and said second pair resulting in a change in at least one detectable property in said sensor.

2. A sensor for the detection of an analyte in a test medium, the analyte being a first member of a pair forming group the sensor comprising:

a matrix having a plurality of porous cavities a single cavity entrapping within it essentially a single detecting pair; wherein the pore properties of the cavities are such that the molecules of the detecting pair are entrapped within the cavities, while the analyte may move freely in and out of the cavities; one molecule of the detecting pair comprises the second member of the pair forming group, and the other molecule of the detecting pair comprises an analyte–analogue which can specifically bind to said second member in a reversible manner; specific binding between the analyte and said second member giving rise to the formation of a first pair and specific binding between said analyte–analogue and said second member giving rise to the formation of a second pair; transition between said first pair and said second pair resulting in a change in at least one detectable property in said sensor.

3. A sensor according to Claim 1 or 2, wherein the matrix is prepared by a sol-gel process involving low-temperature hydrolysis of monomeric precursors of the matrix.

4. A sensor according to Claim 3, wherein the matrix is silicate glass.

5. A sensor according to Claims 1 to 4, wherein the change of at least one detectable property is optical, and wherein said matrix is optically transparent.

6. A sensor according to Claim 5, wherein the optical property is luminescence.

7. A sensor according to Claims 1 to 4, wherein the change of at least one detectable property is electrochemical.

8. A sensor according to Claims 1 to 7, wherein the analyte is an hapten, an antigen, or an antibody-binding-fragment of an antigen.

9. A sensor according to Claims 1 to 7, wherein the analyte is the antibody or an antigen-binding-fragment of an antibody.

10. A sensor according to any of the previous claims, comprising at least one signal producing moiety, said moiety transducing transition from the first pair to the second pair to a change in at least one detectable property.

11. A sensor according to Claim 10, wherein said signal producing moiety is capable of charging its electro-optical properties.

12. A sensor according to Claim 10, wherein said signal producing moiety is capable of charging its electrochemical properties.

13. An apparatus for measuring the concentration of an analyte in a test sample comprising the sensor of any one of Claims 1 to 12, and a detector capable of determining the change of said at least one detectable property.

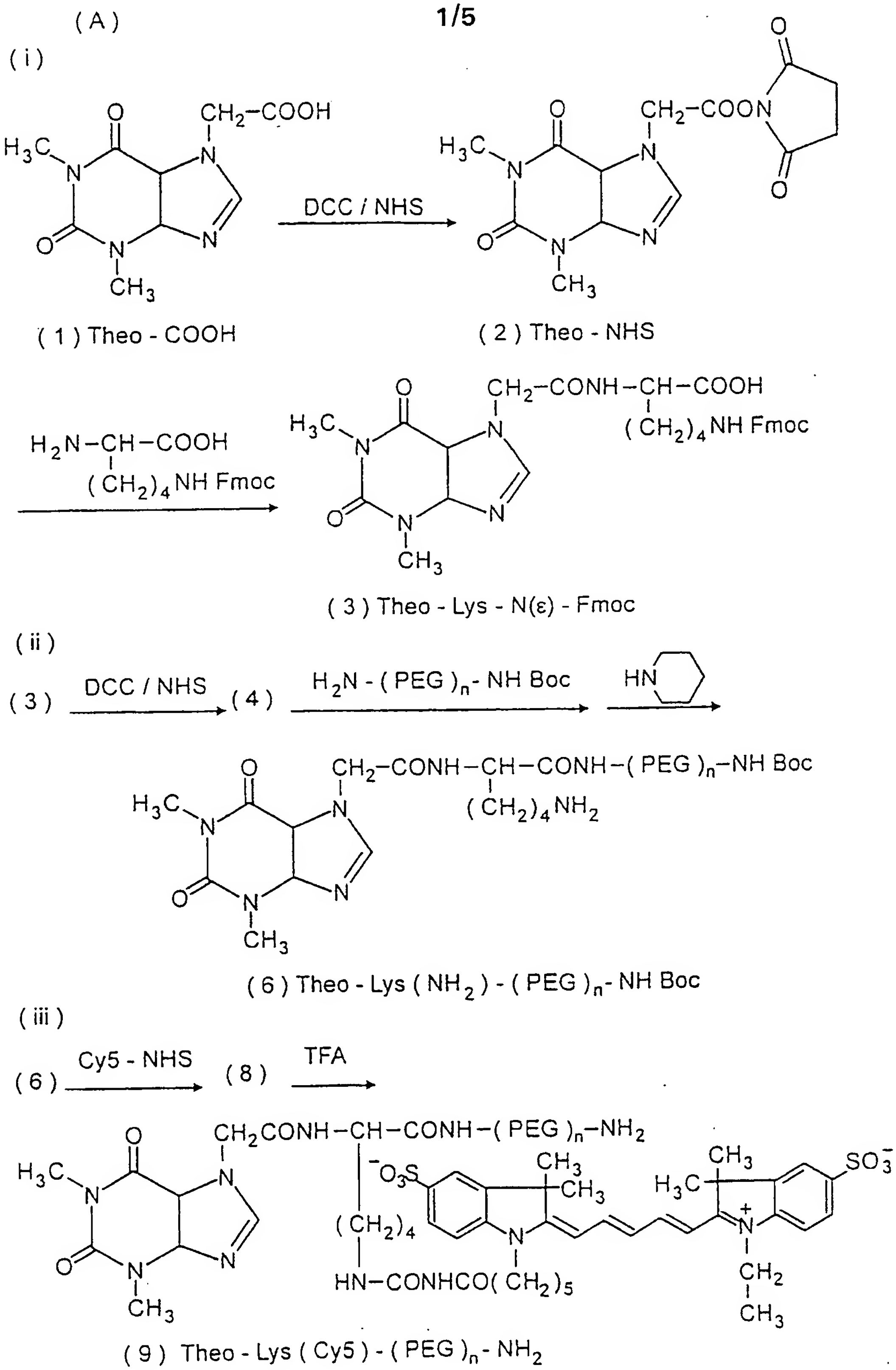
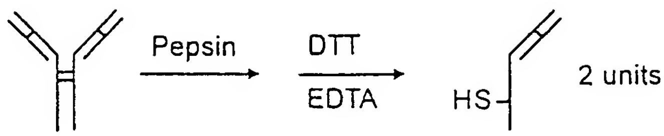

(A)
(i)
DCC / NHS
(1) Theo - COOH
(2) Theo - NHS
(3) Theo - Lys - N(ε) - Fmoc
(ii)
DCC / NHS
H_2N - (PEG)$_n$- NH Boc
(6) Theo - Lys (NH_2) - (PEG)$_n$- NH Boc
(iii)
Cy5 - NHS
TFA
(9) Theo - Lys (Cy5) - (PEG)$_n$- NH_2

Fig. 1

(B)

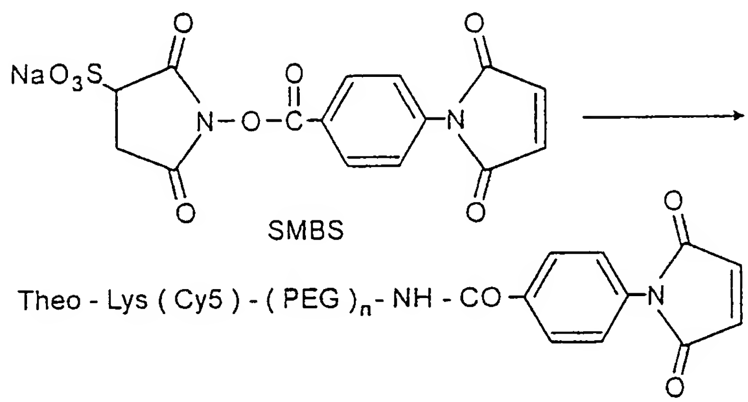


IgG (anti - Theo) (10) Fab (Theo)

(C)

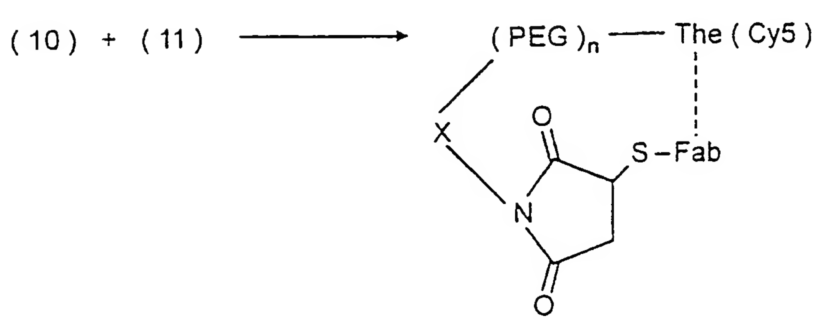
(i)

(8) +



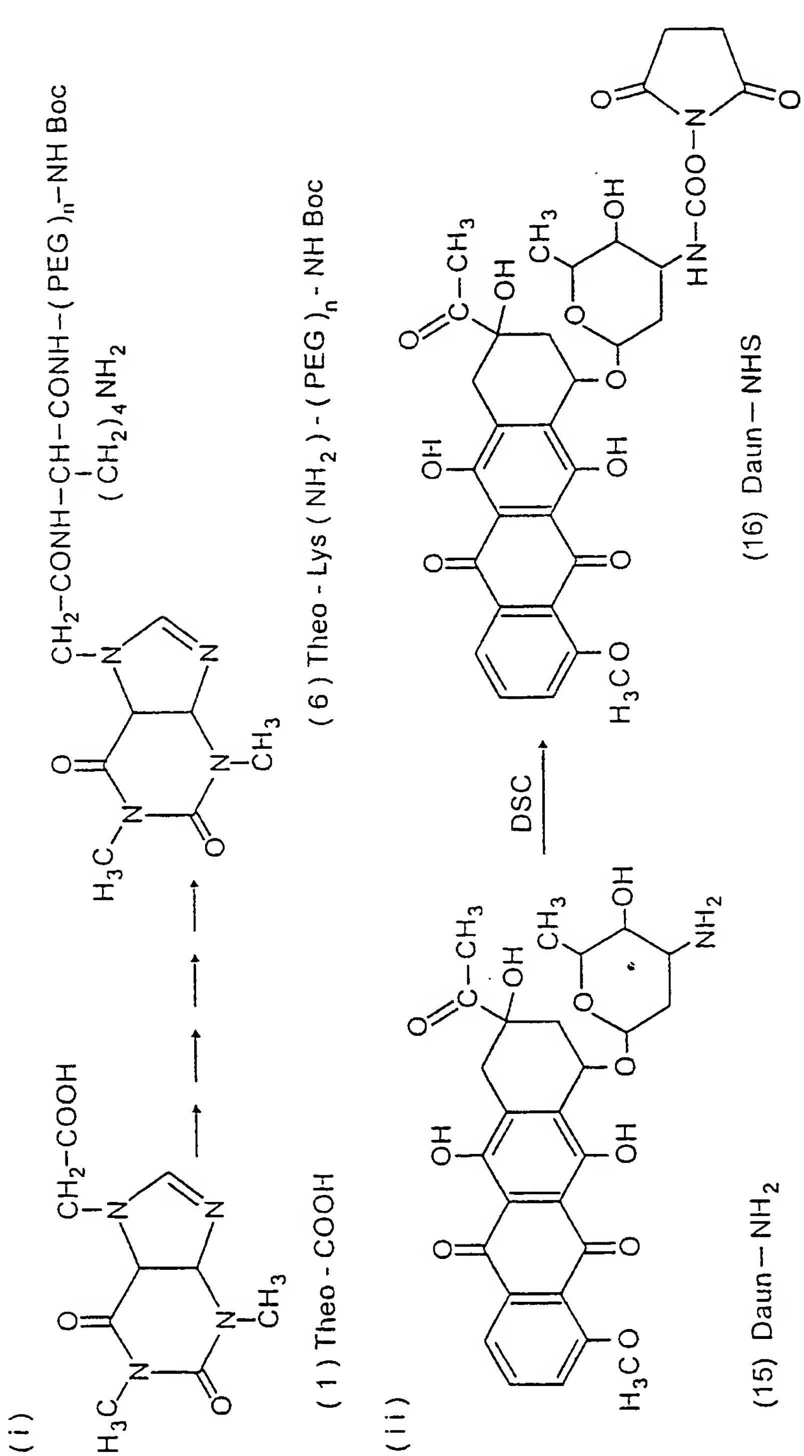
(11)

(ii)



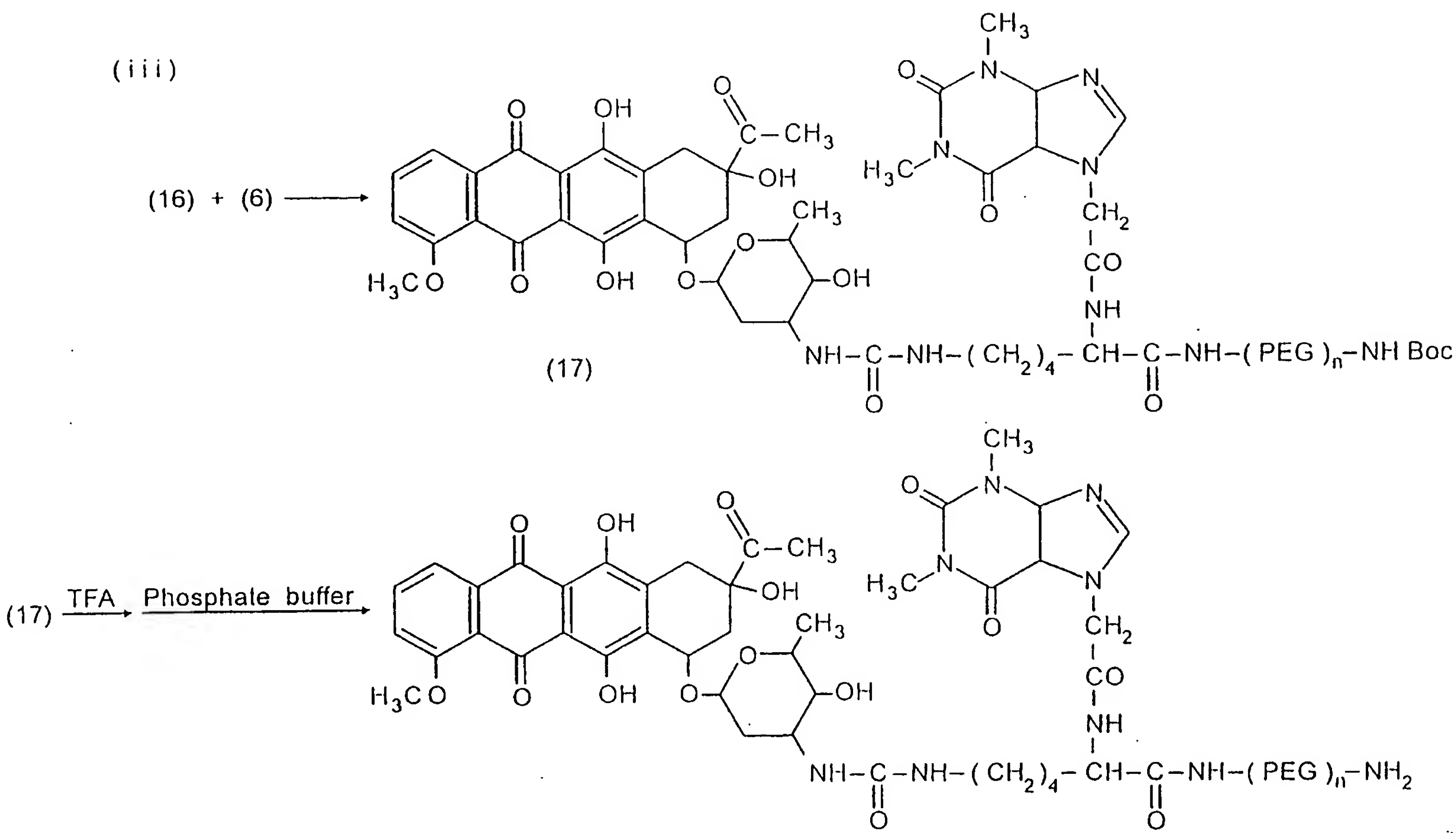
(12) Fab -Theo (Cy5)

Fig. 2


(i)
(1) Theo - COOH
(6) Theo - Lys (NH2) - (PEG)n - NH Boc
(i i)
DSC
(15) Daun – NH2
(16) Daun – NHS

F I G . 3

(iii)



(18) Theo - Lys - (Daun) - $(PEG)_n$ - NH_2

Fig. 3 (cont.)

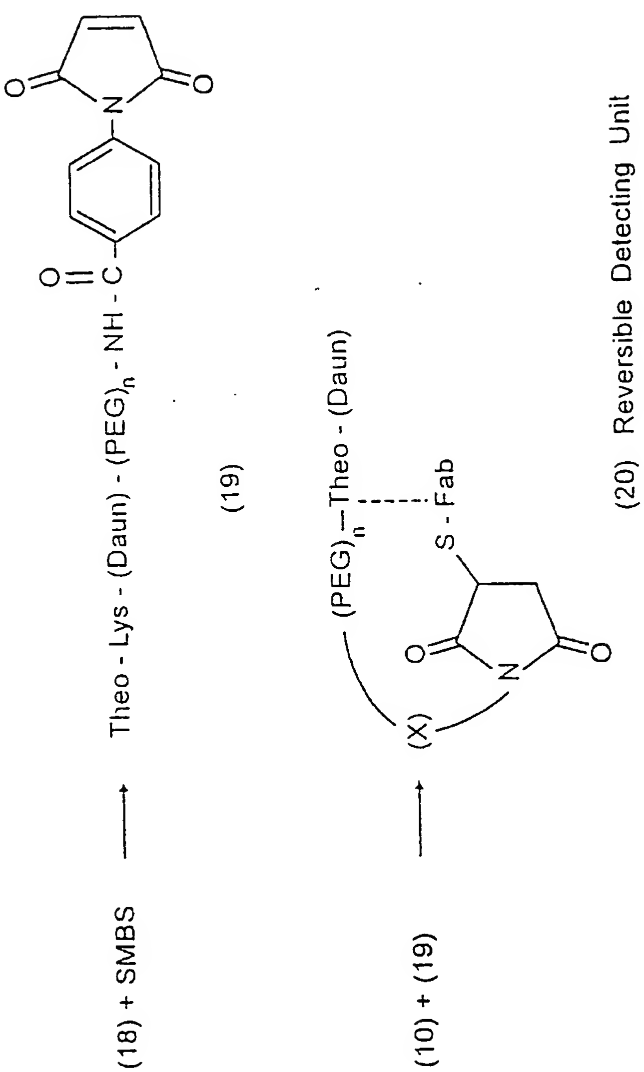

(18) + SMBS
Theo - Lys - (Daun) - (PEG)n - NH - C
O
N
O
O
(19)
(10) + (19)
(PEG)n—Theo - (Daun)
S - Fab
X
N
O
O
(20) Reversible Detecting Unit

Fig. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 97/00221

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 87 03690 A (MUREX CORP) 18 June 1987 see the whole document ---	1,2,13
X	E. H. LAN ET AL: "Heme proteins encapsulated in sol-gel derived silica glasses and their reaction with ligands." MATER. RES. SOC. SYMP. PROC. (BIOMOLECULAR MATERIALS BY DESIGN), vol. 330, 1994, pages 289-294, XP002046717 see the whole document ---	1-4

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
14 November 1997	21.11.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreno, C

INTERNATIONAL SEARCH REPORT

Inte 'onal Application No
PCI/IL 97/00221

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	R. WANG ET AL: "Affinity of antifluorescein antibodies encapsulated within a transparent sol-gel glass." ANALYTICAL CHEMISTRY., vol. 65, 1993, COLUMBUS US, pages 2671-2677, XP002046718 cited in the application see the whole document ---	1-6
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P,X	F. AKBARIAN ET AL: "Spectroscopic determination of cholinesterase activity and inhibition in sol-gel media." J. SOL-GEL SCI TECHNOL., vol. 8, no. 1/2/3, 1997, pages 1067-1070, XP002046720 see the whole document ---	1,2
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A	US 5 156 972 A (ISSACHAR DAVID) 20 October 1992 cited in the application see the whole document ---	1,2
A	US 5 200 334 A (DUNN BRUCE S ET AL) 6 April 1993 see the whole document -----	1,2

3

INTERNATIONAL SEARCH REPORT

..formation on patent family members

Interr nal Application No
PCT/IL 97/00221

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		WO 9304196 A	04-03-93

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